High-Dose Ultraviolet A1 (UVA1), but Not UVA/UVB Therapy, Decreases IgE-Binding Cells in Lesional Skin of Patients with Atopic Eczema

Jürgen Grabbe,* Pia Welker,* Stefanie Humke,†‡ Markus Grewe,†‡ Erwin Schöpf,† Beate M. Henz,* and Jean Krutmann†‡

*Departments of Dermatology, Virchow Clinics, Humboldt University, Berlin, Germany; †University of Freiburg, Freiburg, Germany; and ‡Clinical and Experimental Photodermatology, Department of Dermatology, University of Düsseldorf, Düsseldorf, Germany

In order to further elucidate the mechanisms by which high-dose ultraviolet A1 (UVA1) therapy leads to improvement in patients with atopic eczema, we assessed skin sections from patients before and after high-dose UVA1 therapy (n = 5) or conventional UVA/UVB therapy (n = 4) for changes in Langerhans cells and mast cells expressing the high-affinity IgE receptor FcεRI and in surface-bound IgE by histochemical and immunohistochemical techniques. The two treatment groups exhibited different patterns of changes in the number of FcεRI+, CD1a+, and mast cells within the dermis: The density of both Langerhans cells and mast cells was decreased after high-dose UVA1 therapy, but not after UVA/UVB therapy. High-dose UVA1 and UVA/UVB therapy significantly increased the number of CD1a+ cells within the epidermis, but only high-dose UVA1 reduced the relative number of IgE+ intraepidermal Langerhans cells typically found in atopic eczema. Reduction of numbers of dermal Langerhans cells and mast cells, as well as relative numbers of intraepidermal IgE+ Langerhans cells, was closely linked to significant clinical improvement by high-dose UVA1, but not UVA/UVB therapy. These studies support the notion that IgE-binding cutaneous cells are involved in the pathogenesis of atopic eczema. We propose that UVA1 radiation exerts its effects in atopic eczema, at least in part, by inhibiting Langerhans cell migration out of the epidermis and, in particular, by reducing the number of IgE-bearing Langerhans cells and mast cells in the dermis. *Key words: Langerhans cells/mast cells/FcεRI. J Invest Dermatol 107:419–422, 1996*

The finding that Langerhans cells (LC), in addition to mast cells, bind IgE through the high-affinity receptor FcεRI (Bruynzeel-Koomen *et al*, 1986; Bieber *et al*, 1992; Wang *et al*, 1992; Grabbe *et al*, 1993) prompted speculation about the pathophysiologic role of IgE-bearing LC in atopic eczema. This hypothesis is based on the clinical observation that environmental allergens such as house dust mites or pollen can cause eczematous skin reactions in atopics (Clark and Adinoff, 1989), and on *in vitro* data concerning IgE-mediated antigen presentation (Mudde *et al*, 1990). In addition, mast cells, long known to express FcεRI, have also been identified as the source of several cytokines (Möller and Czarnetzki, 1994), suggesting that they are capable of modulating immune reactions in the skin upon stimulation by receptor-bound IgE after contact with allergens. Recently, it has been shown that high-dose UVA1 (340–400 nm) treatment improves acute exacerbations of atopic eczema more effectively than traditional combined UVA/UVB therapy (Krutmann *et al*, 1992). In order to better understand the UVA1-induced therapeutic effects and the pathophysiology of atopic eczema, we have studied changes in FcεRI- and IgE-bearing LC and mast cells in skin biopsies from patients with atopic eczema before and after UVA1 or UVA/UVB.

Manuscript received March 25, 1995; revised May 15, 1996; accepted for publication May 23, 1996.

Reprint requests to: Dr. Jürgen Grabbe, Department of Dermatology, Virchow Clinics, Humboldt University, Augustenburger Platz 1, D-13344 Berlin, Germany.

Abbreviations: CAE, chloroacetate esterase.

MATERIALS AND METHODS

Patients and Treatment Patients hospitalized for acute exacerbation of severe atopic eczema were allocated to treatment with either high-dose UVA1 (five patients) or conventional UVA/UVB (four patients) irradiation. Details about entrance criteria and treatment of these patients have been published elsewhere (Krutmann *et al*, 1992). The two groups were well matched demographically and clinically, and their clinical scores were comparable (Costa *et al*, 1989; Krutmann *et al*, 1992). Median serum IgE levels were similar (UVA1 group: 1084 U/liter; UVA/UVB group: 1126 U/liter) and did not change significantly under either regimen. After informed consent, biopsies were taken from elbow flexures before therapy and after conclusion of treatment and were immediately snap-frozen and stored at −60°C until processing. The daily dose of UVA1 was 130 joules/cm^2, while doses of UVA/UVB were progressively increased to a mean final dose of 7 joules UVA/cm^2 and 0.028 joules UVB/cm^2. The total number of treatments was 15 for each patient.

Staining Procedures and Evaluation Cryostat sections of biopsies were stained with toluidine blue at pH 0.5 overnight for metachromasia (Schadendorf *et al*, 1995) and by enzymecytochemical methods for tryptase (Harvima *et al*, 1988) and chloroacetate esterase (CAE) (Sterry and Czarnetzki, 1982). Immunohistochemical staining was performed by the alkaline phosphatase–anti-alkaline phosphatase technique as previously described (Cordell *et al*, 1984). The following antibodies were used in the study: monoclonal anti-CD1a, reacting with LC (Dako, Glostrup, Denmark); monoclonal 29C6 against the α-chain of the FcεRI, which binds in the absence as well as in the presence of IgE (Riske *et al*, 1991) (kindly

Table I. High-Dose UVA1 Therapy Reduces Clinical Symptoms of Exacerbated Atopic Eczema More Effectively than UVA/UVB Treatment

	Clinical Score[a]	
Treatment Regimen	Before Therapy	After Therapy
UVA1[b]	63.0 ± 5.8	19.6 ± 5.4
UVA/UVB[c]	56.3 ± 6.2	41.1 ± 10.0

[a] Costa score points (mean ± SD) obtained from patients before and after different types of UV treatment; 10 severity criteria and 10 topographic sites were evaluated and scored from 0 (no lesions) to 6 (extremely severe) or 3 (extended lesions), respectively. For each patient, values were added up to give the total clinical score.
[b] n = 5 patients.
[c] n = 4 patients.

provided by J. Hakimi, Nutley, NJ); and polyclonal anti-IgE (Dako). All monoclonal antibodies were of the IgG1 isotype. An IgG1 anti-desmin antibody (Dako), which stained only occasional smooth muscles (arrectores pilorum), served as negative control. Sections were evaluated by counting nucleated stained cells at 1:400 magnification in at least five adjacent microscopic fields using a microscopic grid covering 1/16 mm^2. Staining reactions in epidermis, papillary dermis, within inflammatory infiltrates, around skin appendages, and between collagen fibers were examined separately. Counts were expressed as stained cells per mm^2 and normalized to a value of 1.0 in order to calculate changes after treatment. Differences in changes of IgE+ and CD1a+ epidermal cells observed under each regimen are given as the ratio of mean IgE+ to CD1a+ cells before and after therapy. Statistical analysis of changes was performed with the Wilcoxon matched-pairs signed-ranks test.

RESULTS

The clinical response of patients treated with high-dose UVA1 was better than that of patients treated with UVA/UVB. Total clinical score was reduced to less than one third of pre-treatment values in the patients receiving high-dose UVA1. UVA/UVB treatment resulted in only minor improvement, and clinical response was more variable, as is indicated by a greater range of values in this group **(Table I)**.

Staining of specimens taken before treatment revealed comparable numbers for CD1a+ and FcεRIα+ cells in the epidermis **(Table II, Fig 1*a,c*)**, indicating that most LC expressed the FcεRI, whereas cell-bound IgE was detectable only in about half the cells. Mast cells were absent, because none of the epidermal cells showed metachromasia or tryptase or CAE activity **(Fig 1*e*)**. In the dermis, the CD1a+ and tryptase/CAE+ cells combined approximately equalled the number of FcεRIα+ cells **(Table III)**. Nearly all of these cells also reacted with the anti-IgE antibody. The density of CD1a+ cells, in particular, and also of tryptase/CAE+ cells was higher in the papillary dermis than in the reticular dermis, whereas metachromasia was greater in the reticular dermis **(Table IV)**. In contrast to the almost equal density of cells with tryptase and CAE in the dermis, metachromatic staining with toluidine blue was detected in only a small number of cells. This discrepancy between staining methods, which has already been described (Algermissen *et al*, 1994; Markey *et al*, 1989; Haas *et al*, 1995), was most apparent in the papillary dermis **(Table IV)**.

In the dermis of UVA1-treated skin, both types of cells binding IgE via the FcεRI were reduced. Significant decreases were noted in metachromatic cells and cells expressing CD1a as well as minor reductions in tryptase+ and CAE+ cells **(Table III, Fig 1*b,d,e*)**. Since separate evaluation of cell numbers revealed no significant differences of UVA1-induced changes between various dermal compartments (papillary dermis, deeper inflammatory infiltrates, around skin appendages, and between collagen fibers) only data demonstrating changes in the staining pattern of the whole dermis are shown. In contrast to the results obtained with UVA1, the density of CD1a+, FcεRIα+, and also of IgE+ cells, albeit more variably, was enhanced after UVA/UVB treatment **(Table III)**. This effect of UVA/UVB on CD1a+ cells was even more pronounced in the reticular dermis than in the papillary dermis, whereas changes in staining patterns for other markers were similar in both compartments (not shown). For mast cells, no effect of UVA/UVB could be demonstrated, independent of their anatomical distribution (not shown) and the staining methods used for detection **(Table III)**.

After high-dose UVA1 treatment, biopsy sections exhibited an increase in CD1a+ epidermal cells **(Table II, Fig 1)**. In contrast, no significant increase in the number of cells bearing FcεRIα or IgE

Table II. Total CD1a+, but Not IgE-Binding Epidermal CD1a+ Cells Increase Under High-Dose UVA1 Therapy of Exacerbated Atopic Eczema

		Change[d]	
Marker	Cell Density[a]	UVA1	UVA/UVB
Metachromasia[b]	0	—	—
Tryptase[b]	0	—	—
CAE[b]	0	—	—
CD1a[c]	560 ± 277	2.08 ± 1.24[e]	3.68 ± 2.52[f]
FcεRIα[c]	458 ± 172	1.35 ± 0.36	2.85 ± 2.71[f]
IgE[c]	278 ± 24	0.92 ± 0.38	3.41 ± 2.64[f]

[a] Mean ± SD of positive cells/mm^2 in biopsies from n = 9 patients with acute exacerbations of atopic eczema before treatment. Evaluation of LC and mast cell numbers was performed by histochemical ([b]) and immunohistochemical ([c]) staining.
[d] Numbers before treatment were set at 1.0 and changes in numbers of stained cells/mm^2 are expressed as mean ± SD of increases or decreases of pre-treatment values in n = 5 (UVA1 group) and n = 4 (UVA/UVB group) patients.
[e] $p < 0.05$.
[f] $p < 0.07$.

Table III. High-Dose UVA1, but Not UVA/UVB, Therapy of Atopic Eczema Reduces Both Dermal CD1a+ and Mast Cells Binding IgE via FcεRI

		Change[d]	
Marker	Density[a]	UVA1	UVA/UVB
Metachromasia[b]	65 ± 6	0.31 ± 0.09[e]	0.91 ± 0.22
Tryptase[b]	141 ± 47	0.95 ± 0.31	0.92 ± 0.19
CAE[b]	173 ± 54	0.86 ± 0.22	1.03 ± 0.13
CD1a[c]	165 ± 65	0.56 ± 0.13[e]	3.59 ± 2.08[f]
FcεRIα[c]	346 ± 12	0.84 ± 0.05[e]	1.67 ± 0.26[f]
IgE[c]	293 ± 63	0.82 ± 0.10[e]	2.16 ± 1.37

[a] Mean ± SD of positive cells/mm^2 in biopsies from n = 9 patients with acute exacerbations of atopic eczema before treatment. Evaluation of LC and mast cell numbers was performed by histochemical ([b]) and immunohistochemical ([c]) staining.
[d] Numbers before treatment were set at 1.0 and changes in numbers of stained cells/mm^2 are expressed as mean ± SD of increases or decreases of pre-treatment values in n = 5 (UVA1 group) and n = 4 (UVA/UVB group) patients.
[e] $p < 0.05$.
[f] $p < 0.07$.

Table IV. CD1a+ and Mast Cells Equal the FcεRIα+ IgE-Binding Cells in Papillary and Reticular Dermis of Atopic Eczema

Marker	Papillary Dermis	Reticular Dermis
Metachromasia[b]	43 ± 9	75 ± 9
Tryptase[b]	182 ± 42	122 ± 43
CAE[b]	227 ± 64	154 ± 49
CD1a[c]	461 ± 218	66 ± 19
FcεRIα[c]	528 ± 204	288 ± 77
IgE[c]	576 ± 189	195 ± 41

[a] Evaluation of LC and mast cell numbers was performed by histochemical ([b]) and immunohistochemical ([c]) staining of skin biopsies from nine patients. Cell density is expressed as mean ± SD of numbers of positive cells/mm^2. (Positive cells within deeper inflammatory infiltrates, around skin appendages, and between collagen fibers were added up giving the average numbers of reactive cells in the reticular dermis.)

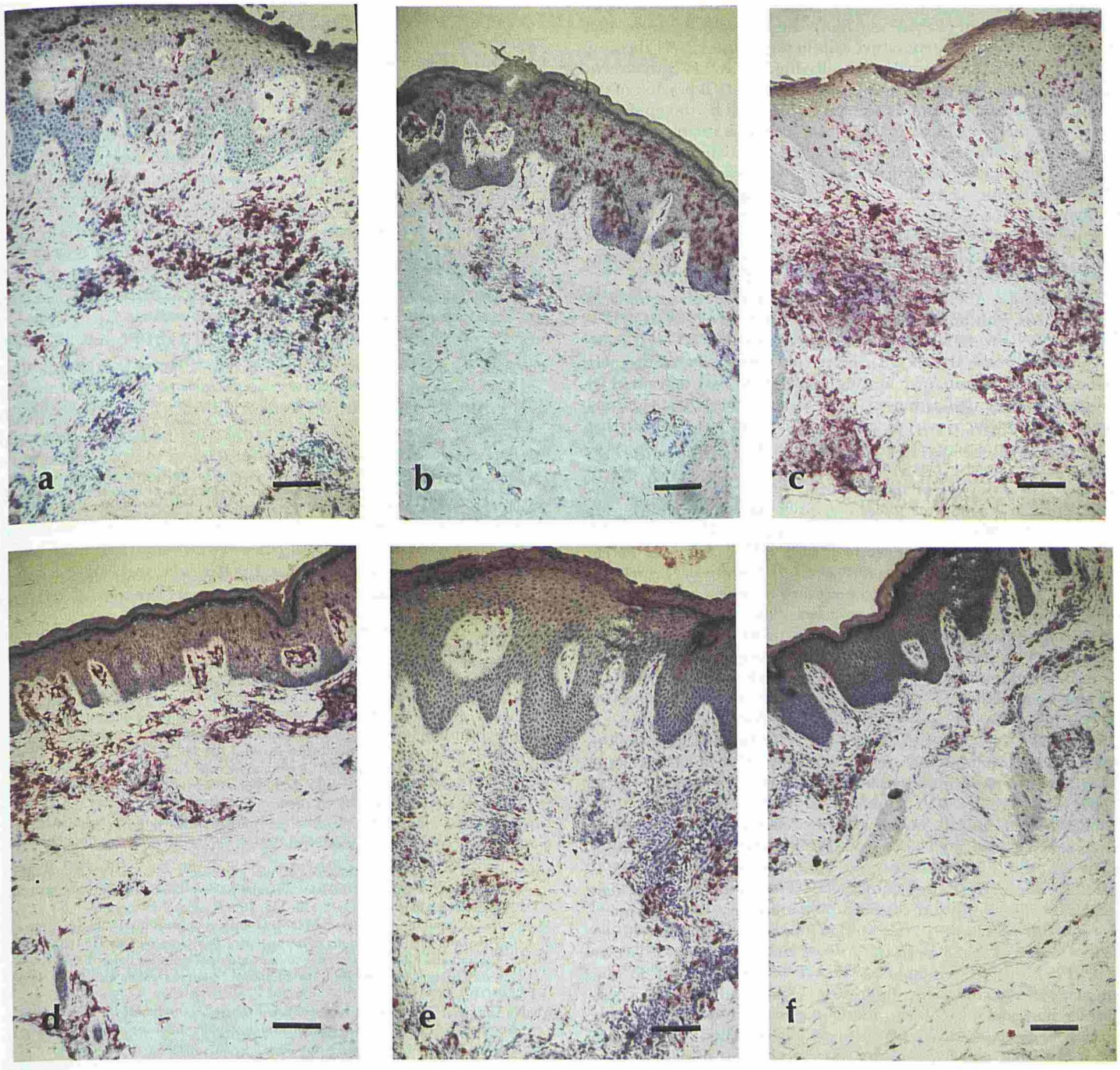

Figure 1. Density of FcεRI-bearing LC and mast cells changes under high-dose UVA1 therapy of exacerbated atopic eczema. Immunohistochemical staining (alkaline phosphatase–anti-alkaline phosphatase system) for CD1a *(a,b)* and FcεRIα *(c,d)* and enzyme histochemistry for CAE *(e,f)* of biopsies from lesions of one patient with atopic eczema taken before *(a,c,e)* and after *(b,d,f)* treatment with high-dose UVA1 (*scale bar*, 100 μm).

could be observed **(Fig 1*d*)**. After UVA/UVB therapy, numbers of LC were also increased, but UVA/UVB and high-dose UVA1 produced different results in relation to the other markers. There was a marked increase also in the number both of FcεRIα+ and IgE+ cells after UVA/UVB **(Table II)**. Thus, despite the increase in absolute numbers of CD1a+ epidermal cells, the ratio of IgE+ to CD1a+ cells decreased in the UVA1 group by a factor of 0.5, whereas it remained nearly constant with minimal change by a factor of 0.94 in the UVA/UVB group.

DISCUSSION

Our data indicate that UVA1 radiation leads to three major changes in IgE-bearing cells in the skin of atopic eczema patients: (i) decrease in metachromatic and CD1a+ as well as FcεRIα+ and IgE+ cells in the dermis, (ii) accumulation of CD1a+ cells, i.e., LC, in the epidermal compartment, (iii) concomitant reduction of FcεRIα+ and IgE+ epidermal LC.

UVA1 therapy and UVA/UVB therapy differed markedly with regard to their effects on both mast cells and CD1a+ cells in the dermis. The number of toluidine blue–stained cells in the dermis was decreased in UVA1-irradiated, but not in UVA/UVB-treated patients. Our findings are in agreement with studies of psoralen plus UVA-treated patients with cutaneous mastocytosis showing a reduction in numbers of dermal mast cells (Granerus *et al*, 1981; Kolde *et al*, 1984). Recent observations suggest that in addition to psoralen plus UVA, high-dose UVA1 radiation therapy may be

effective in the treatment of patients with cutaneous mastocytosis (Stege *et al*, 1996). Taken together, these studies indicate that dermal mast cells represent target cells in high-dose UVA1 therapy. The lack of effect on dermal mast cells after UVA/UVB treatment may be explained by its physical properties: the UVB portion of the emission spectrum is almost completely absorbed by the epidermal layers, and the UVA dose reaching the dermis is relatively low as compared to that from high-dose UVA1 radiation. The most striking difference between the two treatments was in the density of CD1a+ dendritic cells in the dermis. A significantly reduced number of CD1a+ dendritic cells was observed only after UVA1 treatment, whereas UVA/UVB therapy led to an increase in these cells. Again, these differences may be attributable to the different physical properties of UVA1 *versus* UVA/UVB radiation.

Accumulation of CD1a+ cells in the epidermal compartment is in agreement with previous studies in which CD1a+ and CD1c+ epidermal cells were found to be increased after UVA1 treatment, whereas MHC class II+ or IgE-reactive cells were not.[1] Phototherapy-induced intraepidermal accumulation of LC may be caused by UV-induced immobilization of these cells within the epidermal compartment, which may reflect an antiinflammatory effect that prevents their interaction with dermal T cells. UV-inducible cytokines may account for this finding (Streilein, 1995). An increase in LC was also observed in patients undergoing UVA/UVB treatment. There was a marked difference, however, between the effects of the two treatments on the number of IgE+ cells: with UVA1 treatment, the density of FcεRIα+ and IgE+ cells was barely affected, and thus the relative number of IgE-bearing LC decreased, while UVA/UVB therapy led to increased numbers of these cells. These different patterns were associated with different outcomes: clinical improvement in UVA1-treated patients and lack of significant improvement in UVA/UVB-treated patients. The fact that the number of epidermal cells expressing FcεRIα and bearing IgE was not increased in UVA1-treated skin, despite upregulation of CD1a+ cells, may indicate functional changes affecting the expression of IgE-binding sites in these cells. Our findings support the assumption that binding of IgE molecules by LC is of pathogenetic importance for the induction and maintenance of atopic eczema (Clark and Adinoff, 1989; Mudde *et al*, 1990).

Possible mechanisms underlying the clinical improvement of atopic eczema after high-dose UVA1 treatment include inhibitory effects on the expression of the adhesion molecule ICAM-1, decreased numbers of infiltrating helper T cells, and, in particular, reduced *in situ* expression of the proinflammatory cytokine interferon-γ and induction of the antiinflammatory, keratinocyte-derived cytokine interleukin-10 (Krutmann and Trefzer, 1992; Grewe *et al*, 1994, 1995). Comparing the histologic changes in our patients, we conclude that high-dose UVA1 radiation may also exert its therapeutic effects by modulating the number and probably function of FcεRI-bearing LC in the skin. In addition, changes in the number and possibly also the functional status of dermal mast cells may contribute to the clinical effects of the treatments. Additional studies will be needed to clarify whether the observed changes are specific for high-dose UVA1 radiation or may also be observed in healing of atopic eczema, either by spontaneous improvement or in response to other kinds of treatment.

[1] Krutmann J, Sinn S, Czech W, Diepgen T, Simon JC, Kapp A, Luger TA, Schöpf E: Modulation of epidermal Langerhans cell surface markers by high-dose UVA1-therapy. *J Invest Dermatol* 99:114S, 1992 (abstr.).

The excellent technical assistance of Mrs. E. Bauer and Mrs. R. Nordheim is gratefully acknowledged. Work presented in this study was supported by grants from the Deutsche Forschungsgemeinschaft Cz 22/8-1 and Kr 871/3-3.

REFERENCES

Algermissen B, Bauer F, Schadendorf D, Czarnetzki BM: Analysis of mast cell subpopulations (MC_T, MC_{TC}) in cutaneous inflammation using novel enzyme histochemical staining techniques. *Exp Dermatol*, 3:290–297, 1994

Bieber T, De La Salle H, Wollenberg A, Hakimi J, Chizzonite R, Ring J, De La Salle C: Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (FcεRI). *J Exp Med* 175:1285–1290, 1992

Bruynzeel-Koomen CAFM, van Wichen DF, Toonstra J, Berrens L, Bruynzeel PLB: The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. *Arch Dermatol Res* 278:199–205, 1986

Clark RAF, Adinoff AD: Aeroallergen contact can exacerbate atopic dermatitis: patch tests as a diagnostic tool. *J Am Acad Dermatol* 21:863–869, 1989

Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, Macdonald S, Pulford KA, Stein H, Mason DY: Immmuno-enzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP-complexes). *J Histochem Cytochem* 32:219–229, 1984

Costa C, Rilliet A, Nicolet M, Saurat J-H: Scoring atopic dermatitis: the simpler the better? *Acta Derm Venereol (Stockh)* 69:41–45, 1989

Grabbe J, Haas N, Kolde G, Hakimi J, Czarnetzki BM: Demonstration of the high-affinity IgE-receptor on human Langerhans cells in normal and diseased skin. *Br J Dermatol* 129:120–123, 1993

Granerus G, Roupe G, Swanbeck G: Decreased urinary histamine metabolite after successful PUVA treatment of urticaria pigmentosa. *J Invest Dermatol* 76:1–3, 1981

Grewe M, Guyfko K, Krutmann J: Interleukin-10 production by cultured human keratinocytes: regulation by ultraviolet A1 radiation. *J Invest Dermatol* 104:3–6, 1995

Grewe M, Gyufko K, Schöpf E, Krutmann J: Lesional expression of interferon-γ in atopic eczema. *Lancet* 343:25–26, 1994

Haas N, Hamann K, Grabbe J, Algermissen B, Czarnetzki BM: Phenotypic characterization of skin lesions in urticaria pigmentosa and mastocytomas. *Br J Dermatol* 287:242–248, 1995

Harvima IT, Naukkarinen A, Harvima RJ, Fräki JE: Immunoperoxidase and enzyme-histochemical demonstration of human skin tryptase in cutaneous mast cells in normal and mastocytoma skin. *Arch Dermatol Res* 280:363–370, 1988

Kolde G, Frosch PJ, Czarnetzki BM: Response of cutaneous mast cells to PUVA in patients with urticaria pigmentosa: Histophotometric, ultrastructural, and biochemical investigations. *J Invest Dermatol* 83:175–178, 1984

Krutmann J, Czech W, Diepgen T, Niedner R, Kapp A, Schöpf E: High-dose UVA1 therapy in the treatment of patients with atopic dermatitis. *J Am Acad Dermatol* 26:225–230, 1992

Krutmann J, Trefzer U: Modulation of the expression of intercellular adhesion molecule-1 (ICAM-1) in human keratinocytes by ultraviolet (UV) radiation. *Springer Semin Immunopathol* 13:333–344, 1992

Markey AC, Churchill LJ, MacDonald DM: Human cutaneous mast cells—a study of fixative and staining reactions in normal skin. *Br J Dermatol* 120:625–631, 1989

Möller A, Czarnetzki BM: Epidermal cytokines and mast cells. In: Luger TA, Schwarz T (eds.) *Epidermal Growth Factors and Cytokines*. Marcel Dekker Inc., New York, 1994, pp 377–394

Mudde GC, Van Reijsen FC, Boland GJ, De Gast GC, Bruynzeel PLB, Bruynzeel-Koomen CAFM: Allergen presentation by epidermal Langerhans' cells from patients with atopic dermatitis is mediated by IgE. *Immunology* 69:335–341, 1990

Riske F, Hakimi J, Mallamaci M, Griffin M, Pilson B, Tobkes N, Lin P, Danho W, Kochan J, Chizzonite R: High affinity human IgE receptor (FcεRI). *J Biol Chem* 266:11245–11251, 1991

Schadendorf D, Kohlmus C, Gawlik C, Suter L, Czarnetzki BM: Mast cells in melanocytic tumors. *Arch Dermatol Res* 287:452–456, 1995

Stege H, Schöpf E, Ruzicka T, Krutmann J: High-dose UVA1 for urticaria pigmentosa. *Lancet* 347:64, 1996

Sterry W, Czarnetzki BM. *In vitro* differentiation of rat peritoneal macrophages into mast cells: an enzymecytochemical study. *Blut* 44:211–220, 1982

Streilein JW: Immunogenetics of sunlight-induced cancer. In: Krutmann J, Elmets CA (eds.) *Photoimmunology*. Blackwell Science Ltd., Oxford, U.K., 1995, pp 128–152

Wang B, Rieger A, Kilgus O, Ochiai K, Maurer D, Födinger D, Kinet J-P, Stingl G: Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI. *J Exp Med* 175:1353–1365, 1992